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Effect of calcitonin in early and late stages of experimentally induced osteoarthritis. A histomorphometric study

N. A. Papaioannou M.D., Ph.D., Associate Professor of Orthopaedics†,
I. K. Triantafillopoulos M.D., Ph.D., Orthopaedic Surgeon†*,
L. Khaldi M.D., Consultant Pathologist†‡, N. Krallis M.D., Orthopaedic Surgeon†,
A. Galanos M.Sc., Biostatistician† and G. P. Lyritis M.D., Ph.D., Associate Professor of Orthopaedics†
† *Laboratory for the Research of the Musculoskeletal System, Medical School, University of Athens, Athens, Greece*
‡ *Department of Pathology, University Hospital of Larissa, Larissa, Greece*

Summary

Objective: To investigate both prophylactic and therapeutic roles of salmon calcitonin on the articular cartilage of rabbit's knees.

Methods: Right knee instability was produced in 30 New Zealand white rabbits by sectioning the cranial cruciate ligament (CCL). Animals were separated into four groups: placebo prophylactic-stage group ($n=6$), killed 8 weeks post surgery, calcitonin prophylactic-stage group ($n=6$), treated immediately after surgery with salmon calcitonin and killed at 8 weeks, placebo therapeutic-stage group ($n=9$) killed at 16 weeks post surgery and calcitonin therapeutic-stage group ($n=9$), treated with salmon calcitonin from 8th to 16th week and killed at 16 weeks post surgery. A histomorphometric study was based on the morphological changes of the articular cartilage and subchondral bone (degeneration indexes), as well as the articular cartilage thickness, chondrocytes' arrangement and their metabolic activity (regeneration indexes).

Results: Calcitonin groups showed smoother articular surface, no or minimal signs of ulceration, smaller osteophytes, and less subchondral cystic formation than placebo groups. Normal distribution of chondrocytes or hypercellularity was noticed in areas of mild osteoarthritic (OA) changes in the calcitonin groups indicating regeneration activity. Periodic Acid Schiff's and Alcian blue staining were negative in the placebo groups while increased absorption in the calcitonin groups revealed high anabolic activity.

Conclusions: In prophylactic stages salmon calcitonin seemed to inhibit the progression of osteoarthritis by increasing the layers of hyaline cartilage, restoring the cellular metabolism, and decreasing the volume of osteophytes. In therapeutic stages, the hormone had a healing effect by decreasing the subchondral cysts, regenerating the hyaline cartilage and restoring cellular metabolism. Both macroscopic and histological findings of this study supported the biochemical results of previous studies showing the therapeutic effect of calcitonin on osteoarthritis.

Key words: Calcitonin, Osteoarthritis, Animal studies, Anterior cruciate ligament Transection, Histology.

Introduction

Conservative treatment regimens for osteoarthritis treat the symptoms but not the disease and are limited to control pain and inflammation and eliminate the risk factors[1,2]. Furthermore, it is still debated if simple analgesics are effective as oral drugs of first choice[3,4]. Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are associated with serious adverse events particularly gastrointestinal. Selective cyclo-oxygenase-2 (COX-2) inhibitors reduce the incidence of upper gastro-intestinal tract ulcerations, however, other toxicities such as fluid retention, hypertension, congestive heart failure, renal insufficiency and a risk for cardiovascular thrombosis may occur[5,6]. The therapeutic role of high molecular weight hyaluronans, chondroitin sulfate and glucosamine in terms of pain relief and slowing the progression of osteoarthritis is also debated[7–9]. Finally, arthroplasty for end stage disease process has a limited life span, relieves pain more predictably than it improves joint function and can be associated with local and systematic complications[10].

Seeking other pharmaceutical interventions, calcitonin may be a potential agent for the treatment of osteoarthritis. Previous data indicated calcitonin's safety and effectiveness on reduction of bone turnover[11] and it's analgesic effect in relieving osteoarthritic (OA) pain[12,13]. Furthermore, there is *in vivo* and *in vitro* experimental evidence that calcitonin acts on both cartilage and subchondral bone by decreasing the enhanced turnover of the OA subchondral bone, reducing the severity of cartilage OA lesions and altering the biochemical composition and supramolecular organization of the OA cartilage matrix[14,15].

The purpose of this study was to evaluate macroscopically and microscopically the effect of salmon calcitonin on the articular cartilage in prophylactic and therapeutic stages of experimentally induced OA in rabbits and to determine the preventive and/or reparative activity of the hormone.

*Address correspondence and reprint requests to: Ioannis K. Triantafillopoulos, M.D., Ph.D., 6 Dimitros Str, 15124 Maroussi, Greece. Tel./Fax: 30-210-6124007; Mob.: 30-6937266639; E-mail: apjt@hol.gr
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Materials and methods

The experimental protocol was approved by the Veterinary Directorate (permit no.1130), according to the Greek Presidential Decree 160/1991, which conforms to the EEC Directive 609/1986 for the "protection of vertebrate animals used for experimental or other scientific purposes". Thirty adult male New Zealand white rabbits (*Oryctolagus cuniculus*), conventionally bred, were used. The animals were obtained from the conventional breeding facilities of the Hellenic Pasteur Institute and housed singly in steel cages of 45 cm × 30 cm × 60 cm dimensions (IFFA CREDO, BP 0109-69592, L'Arbresle, France) in the Laboratory for Research of the Musculoskeletal System conventional animal house. Their age ranged from 4 to 6 months and their weight ranged from 3.4 to 4.9 kg. The age and X-rays determined that the animals were skeletally mature. Additionally, the animals were randomly assigned so that each group had 4, 5 and 6 months old animals in them. The temperature therein ranged between 18 and 21°C, relative humidity 50–60%, the light/dark cycle was from 06:00 to 18:00 and there were 15 air changes/hour. The animals' body weight was 2040 ± 200 g (mean ± SD). They had free access to standard rabbit pellets (14% protein, 7% fat, 15% cellulose, 1–1.2% calcium and phosphorus) (ELVIZ Hellenic Feedstuffs Ind. SA, Plati, Imathia, Greece) and tap water.

SURGERY

All animals were operated on day 1. After induction of general anesthesia (ketamine 25 mg/kg and midazolam 5 mg/kg intramuscularly), the right stifle was shaved and the skin prepped. Anesthetic depth was monitored throughout the procedure by a DINAMAP vital signs monitor 1846. Through a medial parapatellar incision the cranial cruciate ligament (CCL) was transected macroscopically with a No 11 blade. After transection, anterior instability was manually confirmed by the anterior drawer test. The incision did not disrupt the patellar apparatus and no patellar luxation was needed for the CCL transection. The articular capsule and the medial retinaculum were closed with absorbable sutures and the skin with nylon sutures. After recovery, rabbits received paracetamol suppository (Dolal supp. bebe, 1/3 supp. = 50 mg) for pain relief and were free to move in their cages without any external immobilization. They also received paracetamol syrup (Dolal sir. 1 ml = 25 mg) twice a day the next postoperative day. The animals were checked daily (activity, body weight, food consumption, rectal temperature, wound healing) for signs of ill health for the first postoperative week.

TREATMENT REGIMENS

The animals were separated into four groups depending on the time of euthanasia and the treatment received. The first group, *placebo prophylactic-stage group* included six rabbits that were euthanised at 8 weeks in order to confirm the extension and grade of the developed OA lesions. In the literature, the period between 6 and 8 weeks after sectioning of the CCL is considered sufficient for the development of osteoarthritis in rabbits[16]. The second group, *calcitonin prophylactic-stage group* included six rabbits that received 7 IU salmon calcitonin injected intramuscularly daily from day 1 to week 8 postoperatively and then killed. The third group, *placebo therapeutic-stage group*, included nine rabbits that were placebo treated and euthanised at the sixteenth postoperative week. The fourth group, *calcitonin therapeutic-stage group*, included nine rabbits that received 7 IU salmon calcitonin injected intramuscularly daily from week 8 to week 16 postoperatively and then killed. Euthanasia was carried out by ketamine/midazolam premedication administered intramuscularly, followed by slow intravenous administration of sodium thiopental (20–30 mg/kg until cessation of cardiac function) in normal saline drip.

SPECIMEN COLLECTION

After euthanasia, all specimens were prepared as follows: excision of the skin, osteotomy 3 cm above and below the knee joint and fixation of the specimens in 10% buffered formalin for 24 h. Fixed specimens were cleared from soft tissues and ligaments, allowing the gross examination of the articular surfaces of the femoral condyles and tibial plateaus and charting of the specimens. The specimens were then decalcified in 10% nitric acid for 3 to 5 days. A non-operated control group was not employed for two reasons: (1) no OA or degenerative changes were observed in any of the joints during surgery and (2) rabbits have very little spontaneous degeneration in their knee joints[17].

SPECIMEN EXAMINATION

The macroscopic OA lesions included (1) osteophytes, (2) cartilage erosion (ulceration, fissures) and (3) loss of cartilage luster (softening, fibrillation). The evaluation of these lesions was based on three macroscopic parameters: (1) the location, (2) the type, and (3) the size of the OA changes. Macroscopic evaluation of the specimens included: (1) articular surfaces digital photographing (Nikon F5, Nikon Co, Japan) (×4 objective zoom) and scanning of the negative films (Scanjet 3200C, Hewlett Packard, Palo Alto, CA, USA), (2) the articular changes of the femoral condyles, femoral trochlea, and tibial plateaus were drawn on millimeter paper (×2 magnification), and (3) imprint on carbon paper of the OA changes with the use of ink paper. The area of the articular surface involving these lesions was measured using a Vernier caliper. The articular surfaces of the knee were divided in five sections (I–V) in order to define the areas with the highest incidence of OA alterations and standardize the size of histological sections (Table I).

Table I
Location of the macroscopically observed osteoarthritic (OA) changes in all groups

Site	Articular surface area	Predominant OA lesions
I	Femoral trochlea	Osteophytes
II	Middle of the medial femoral condyle	Severe ulceration and loss of luster
III	Middle of the lateral femoral condyle	Modest ulceration and loss of luster
IV	Anterior part of the medial and lateral tibial plateau	Mild ulceration and loss of luster
V	Posterior part of the medial and lateral tibial plateau	Mild ulceration and loss of luster

The histological sections were performed in the above-mentioned sites and were stained with Haematoxyline/Eosin (H/E), Periodic Acid Schiff's (PAS), and Alcian blue. For the microscopic evaluation, five histological parameters were taken into consideration: (1) morphology of the articular cartilage, (2) morphology of the subchondral bone, (3) thickness of the cartilage (comparison between normal and affected sites), (4) arrangement of chondrocytes into the hyaline cartilage, and (5) metabolic activity of the chondrocytes. The first two microscopic parameters (1, 2) were indicative of degeneration while the last three (3–5) were evidence of a repairing and regeneration process.

In order to establish a more objective demonstration of the histological parameters, a scoring system was used (Table II). This scoring system was based on a modified Mankin's grading system[18]. Every histological parameter was classified according to the severity of the osteoarthritis on a scale from 0 to 5. Cartilage normal in appearance was graded as 0 while the scale went up proportionally to the severity of the OA lesion. The quantified classification of the histological findings was entered into a database and statistical analysis was performed. The microscopic

Table II

The evaluation of the natural history of the induced osteoarthritis was based on five histological parameters scored according to a modified Mankin's grading system[18]

Articular cartilage morphology	
Normal	0
Hypertrophy	1
Ulceration	2
Subchondral bone morphology	
Normal	0
Cyst development	1
Cartilage thickness	
Normal	0
Increase	1
Decrease	2
Arrangement of chondrocytes	
Normal	0
Hypercellularity	1
Aggregation	2
Hypocellularity	3
Metabolic activity and chondrocytes (PAS and Alcian blue staining)	
Positive staining (anabolic stage)	0
Negative staining (catabolic phase)	1

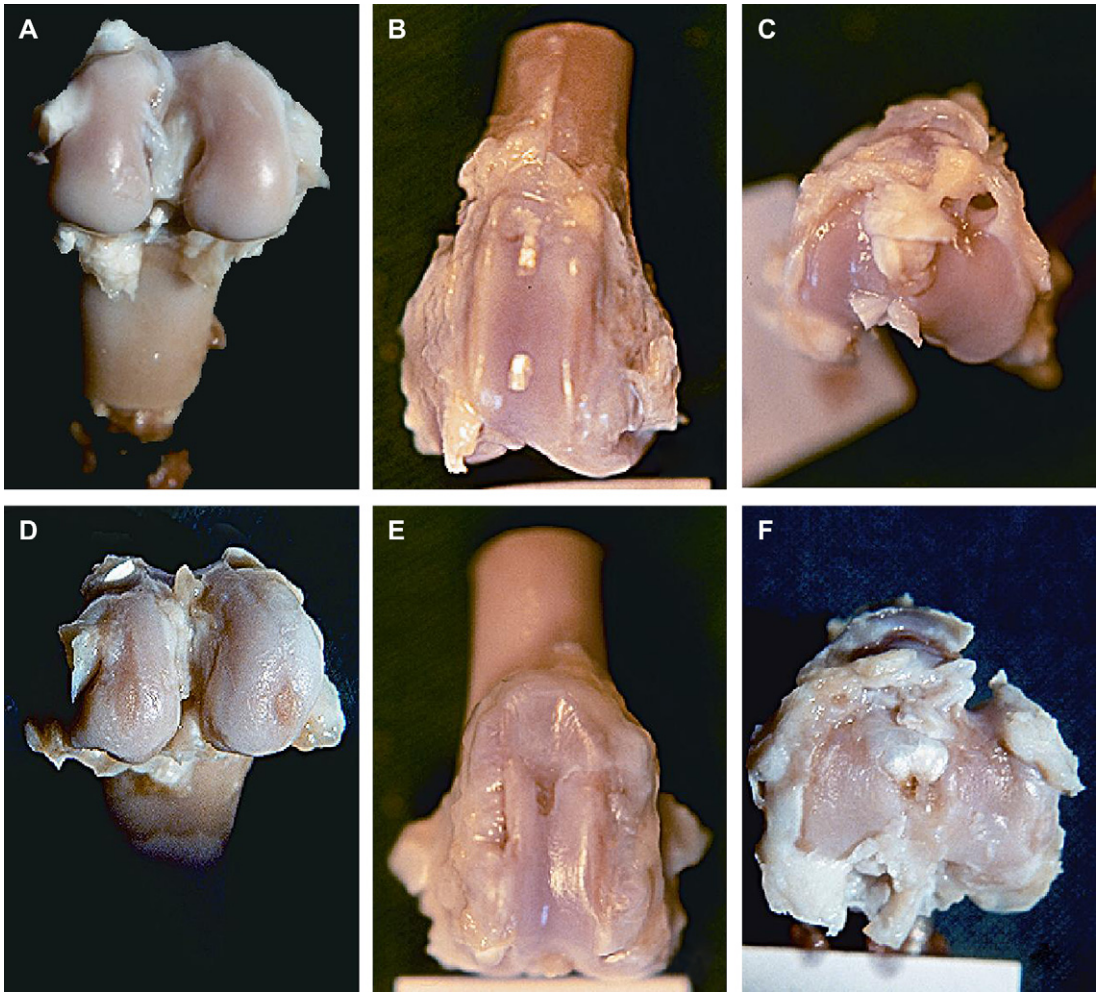

Fig. 1. Macroscopical appearance of a *calcitonin prophylactic-stage group*: femoral condyle (A), femoral trochlea (B), tibial plateau (C), and a *placebo prophylactic-stage group*: femoral condyle (D), femoral trochlea (E), tibial plateau (F).

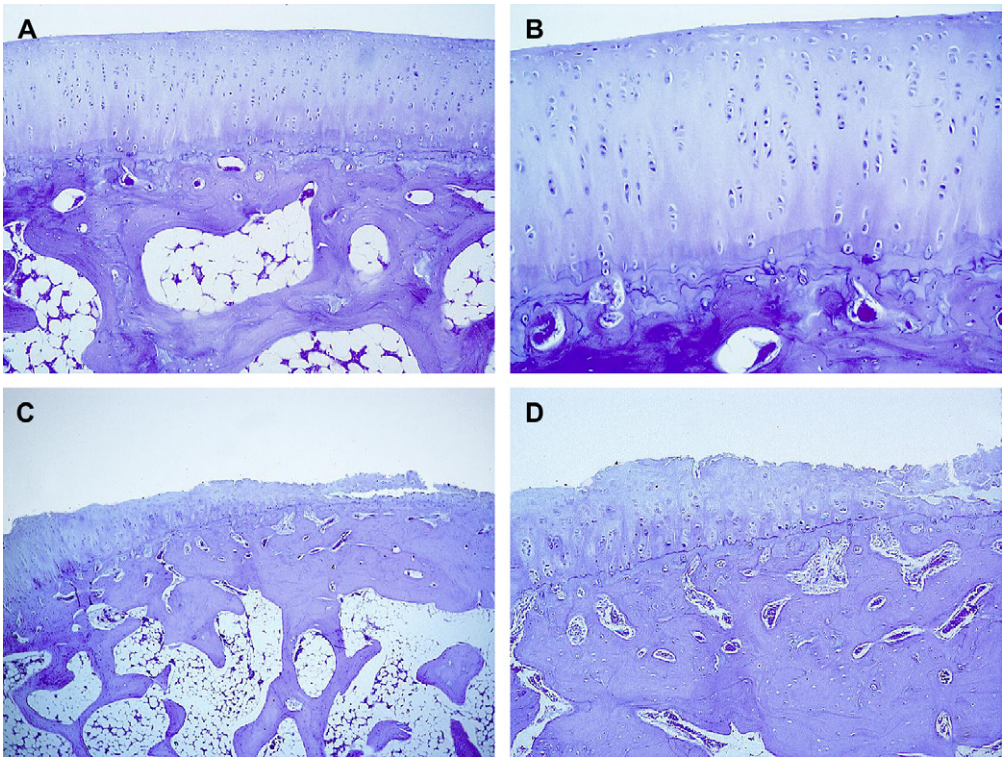

Fig. 2. Microscopical sections of the medial femoral condyle showing histological changes of the articular cartilage in a *prophylactic-stage group*: calcitonin prophylactic-stage 4 (A), calcitonin prophylactic-stage ×10 (B), placebo prophylactic-stage ×4 (C), placebo prophylactic-stage ×10 (D). Notice articular thickness, chondrocyte distribution and subchondral cysts (hematoxylin/eosin staining, ×4 and ×10 magnifications).

measurement of the cartilage thickness was performed under a magnification of ×10 in increments of 0.01 mm real thickness (1 cm of the microscopic scale corresponds to 0.39 mm of real thickness). The total score of all five parameters characterized the severity of the OA changes in every histological section. Adding the score from all histological sections of each animal summed the total score for that animal. The sum of the total scores of all the animals of each group produced the median and the mean values that were used for the statistical analysis of the results.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS statistical software (SPSS Inc, Chicago, IL, USA). Comparisons between groups were performed using the Student's *t* test for the macroscopic parameters (quantitative data) and non-parametric Wilcoxon test was used to analyze the histological parameters (ordinal data). A value of $P < 0.05$ was selected to indicate statistical significance.

Results

OA changes developed in all animals. The predominant macroscopically observed OA changes in all groups were: osteophytes in site I (trochlea), severe ulceration in site II (medial femoral condyle), medium ulceration in site III (lateral femoral condyle), and mild ulceration as well as marginal osteophytes in sites IV and V (tibial plateaus) (Table I).

Macroscopically, both calcitonin groups showed smoother articular surfaces resembling the healthy cartilage, smaller osteophytes and no obvious ulcerations when compared to the placebo groups (Figs. 1 and 2) (Table III). The placebo groups showed fibrillation of the cartilage, larger osteophytes and ulceration of the articular cartilage in the weight bearing areas of both femour and tibia (Figs. 1 and 2) (Table III).

Microscopically, the morphology of articular cartilage in the *calcitonin prophylactic-stage group* showed a more normal appearance in comparison to the *placebo prophylactic-stage group* ($P = 0.002$) (Fig. 3) (Table III). Although those differences were also evident between the

Table III
Macroscopical and microscopical findings showing the differences between the four treatment groups. Percentages express the number of specimens that demonstrated these findings in each group (NS = not significant)

	Prophylactic-stage groups			Therapeutic-stage groups		
	Calcitonin (%)	Placebo (%)	*P* value	Calcitonin (%)	Placebo (%)	*P* value
Osteophytes	37.5	40	NS	46.7	43.3	NS
Ulcerations	12.5	46.7	0.001	13.3	43.3	0.02
Normal cartilage appearance	37.5	8.9	0.002	30	13.3	NS

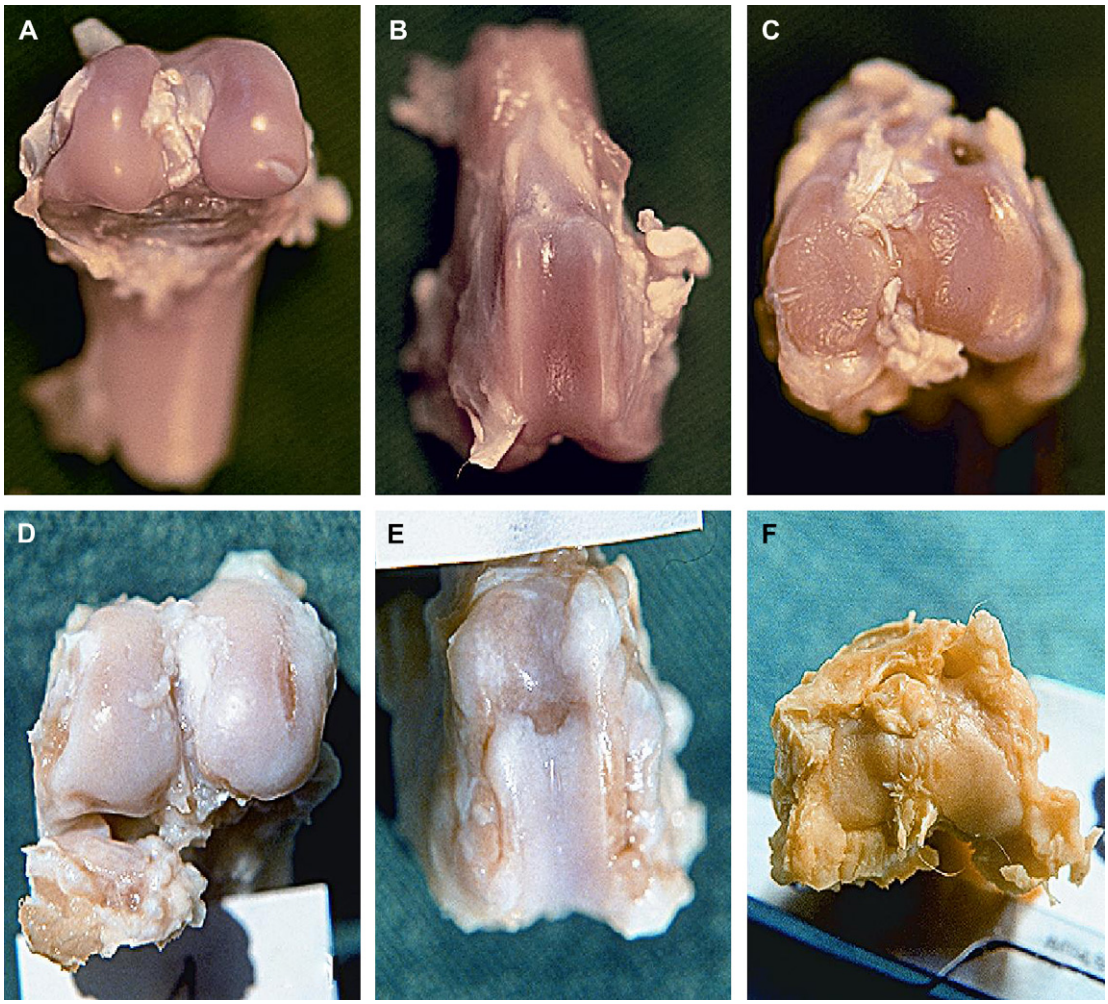

Fig. 3. Macroscopical appearance of a *calcitonin therapeutic-stage group*: femoral condyle (A), femoral trochlea (B), tibial plateau (C) and a *placebo therapeutic-stage group*: femoral condyle (D), femoral trochlea (E), tibial plateau (F).

therapeutic-stage groups, they did not reach statistical significance (Fig. 4) (Table III). In the *calcitonin groups*, rare ulceration and slight increasing of chondrocyte layers were noticed. Furthermore, in the *calcitonin groups*, the osteophytes were smaller in size than the osteophytes in the *placebo groups*. Additionally, the osteophytes in the *calcitonin groups* had a tendency of central reossification (without any evidence of endochondral bone formation) while the osteophytes in the *placebo groups* had a necrotic (non-cellular) central area (Table III). There were statistically significant differences when the size of the articular cartilage and the subchondral cystic formation were compared between groups. Cartilage ulceration and subchondral cysts were more evident in the *placebo groups* (Table IV). Comparison of the articular cartilage thickness in healthy and affected areas did not reveal statistical significance (Table V). However, cartilage was thicker in the *calcitonin groups* than in the *placebo groups*. The distribution of chondrocytes (Table VI) in areas with mild OA lesions was normal in calcitonin groups or slightly hypercellular indicating regenerative activity (median value 0 for prophylactic stage and 2 for therapeutic stage) whereas in the placebo groups, hypocellularity or aggregation of the chondrocytes was found (median value 2). PAS and Alcian blue staining (Table VII), indicating the metabolic activity of the cartilage, was positive in the majority of the *calcitonin group* specimens and in the minority of the *placebo group* specimens. Additionally, in the *calcitonin groups* increased absorption of PAS stain from the nuclei and Alcian blue stain from the cytoplasm indicated the high anabolic phase of the chondrocytes including restoration of mucopolysacharides and acidic-mucopolysacharides in sites of mild OA defects.

Generally, the above described changes were noticed with statistical significance mainly at the lateral femoral condyle (site III) and posterior tibial plateau (site IV) in prophylactic stages (Fig. 5) whereas trochlea (site I) was more affected in therapeutic stages (Fig. 6).

Discussion

It is recently recognized that different joint components contribute to progression of the osteoarthritis[19,20]. Therefore, an ideal medication for its therapy should have a favorable impact on the different aspects of the disease process, including structural and metabolic changes of the joint components. Seeking the ideal drug, calcitonin seems to fulfill many requested criteria such as safety at therapeutic doses[11], analgesia on

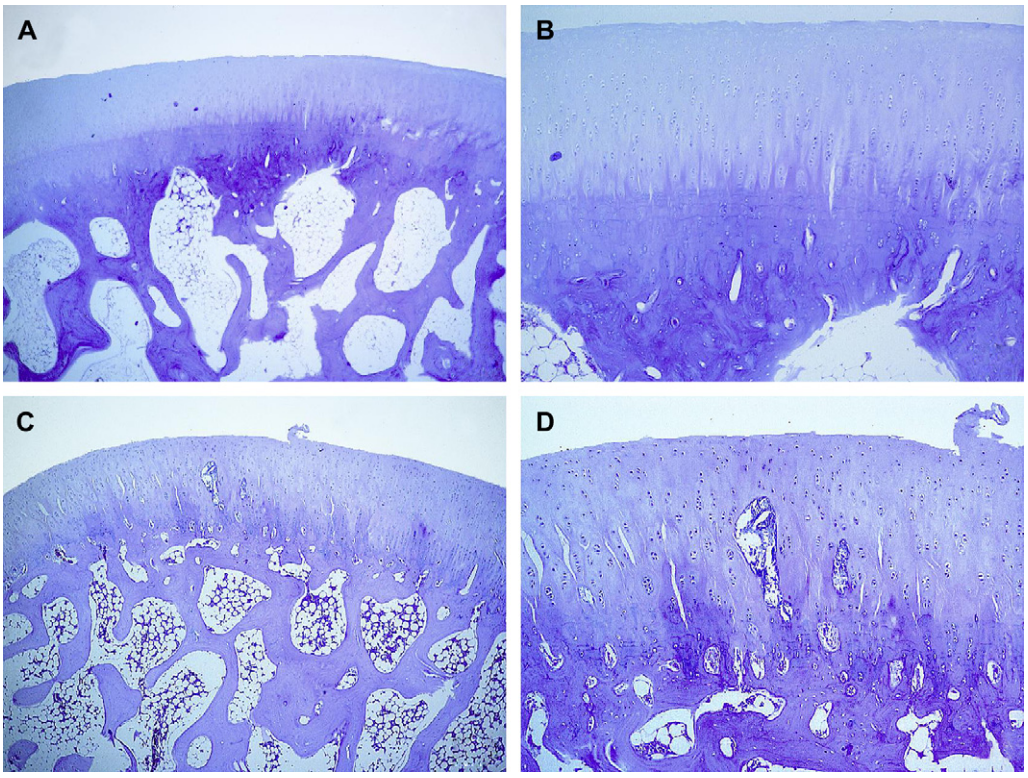

Fig. 4. Microscopical sections of the medial femoral condyle showing histological changes of the articular cartilage in a *therapeutic-stage group*: calcitonin therapeutic-stage ×4 (A), calcitonin therapeutic-stage ×10 (B), placebo therapeutic-stage ×4 (C), placebo therapeutic-stage ×10 (D). Notice articular thickness, chondrocyte distribution and hypochondral cysts (hematoxylin/eosin staining, ×4 and ×10 magnifications).

the clinical management of the disease[12,13,21], anti-inflammatory properties[22–27] and positive effect on the morphology and metabolism of both subchondral bone and articular cartilage[28–30].

The positive effect of calcitonin on bone metabolism is based on histological studies showing that the hormone improves the rate of bone turnover, the organization of trabeculae and the quality of bone matrix deposition in patients with Paget disease[29] and in patients with osteoarthritis[28,30]. This positive effect on bone mineralization and its structural organization improves significantly the biomechanical properties of the subchondral bone tissue[31,32]. Additionally, *in vitro* studies exhibited a stimulatory effect of calcitonin on the cartilage chondrocytes production of proteoglycans and collagen-II[33–35]. Furthermore, calcitonin reduces the collagenase activity present in OA cartilage explants[27,34].

The strongest evidence for calcitonin as a potential agent for the treatment of human osteoarthritis arises from the results of pre-clinical animal studies. In a rabbit model, the hormone hampers the loss of cartilage glycosaminoglycans[35] and in canine models of experimental osteoarthritis, calcitonin prevented periarticular osteopenia, reduced joint inflammation, turnover markers, synovial proliferation markers, aggrecan metabolism markers, and bone resorption markers[14,15]. In another canine experimental osteoarthritis study, calcitonin reduced the hyaluronan and collagen loss associated with the development of OA changes[20] .

The macroscopical and histological findings of this study supported the results of the above experimental studies indicating the chondro-protective and chondro-regenerative roles of salmon calcitonin in experimentally induced

Table IV

*There were statistically significant differences when the size of the articular cartilage (*with interval 0.01 mm of real thickness*) and the subchondral cystic formation were compared between groups. Minimum and maximum values in the parentheses were according to the modified Mankin's grading system[18] and represent the summary from all the sites*

Groups	Minimum and maximum values			
	Chondral morphology (hypertrophy, ulceration)		Subchondral morphology (cysts)	
Calcitonin prophylactic-stage	(3–7)	$P = 0.05$	(3–9)	$P = 0.012$
Placebo prophylactic-stage	(3–12)		(9–15)	
Calcitonin therapeutic-stage	(2–7)	$P = 0.005$	(6–15)	$P = 0.002$
Placebo therapeutic-stage	(5–12)		(12–15)	

Table V
Comparison of the articular cartilage thickness in healthy and affected areas (areas of normal vs osteoarthretic from the same anatomic site which was the medial femoral condyle) (measurements are in mm)

	Prophylactic-stage calcitonin vs placebo	Therapeutic-stage calcitonin vs placebo
Areas of normal appearance	3.9* ± 0.8 vs 3.4* ± 0.6, $P = 0.01$	4.1[a] ± 1.3 vs 4.4[a] ± 0.9, $P =$ NS
Areas of mild OA changes	8.7[a] ± 3.4 vs 7.7[a] ± 2.5, $P =$ NS	9.5[a] ± 1.9 vs 9.3[a] ± 1.2, $P =$ NS
Areas of severe OA changes	1.1[a] ± 0.6 vs 0.6[a] ± 0.4, $P =$ NS	1.2[a] ± 0.9 vs 0.8[a] ± 0.5, $P =$ NS

*Statistically significant when $P < 0.05$.
[a]With interval 0.01 mm of real thickness.

arthritis. The *placebo prophylactic-stage group* confirmed the development of osteoarthritis 8 weeks after the transection of the CCL and the experimentally induced knee instability. A recent study by the same authors showed that a period of 8 weeks after CCL transection was sufficient for the development of OA lesions in rabbit knees[36].

Macroscopically, in the *placebo prophylactic-stage group*, osteophyte formation was predominantly noted at the proximal part of the femoral trochlea (site I) and severe ulceration and loss of luster were prominently noted at medial femoral condyle (site II). The combination of two biomechanical factors may explain the predominant and significant macroscopic OA findings at the trochlea. Firstly, the patello-femoral articulation is the most mobile site of the knee and the friction of the articular cartilage at this area is greater than that in other sites of the joint. Secondly, the flexion of the rabbit's knee at the standing position contributes to the greater loading of the patello-femoral articulation[37]. *Calcitonin prophylactic-stage group* showed a more normal appearance than the *placebo prophylactic-stage group* indicating the chondro-protective role of the hormone. *Calcitonin therapeutic-stage group* showed evidences of cartilage healing with smaller osteophytes than the *placebo therapeutic-stage group*, supporting the repairing (chondro-therapeutic) role of the hormone.

Microscopically, both calcitonin groups showed rare cartilage ulceration and increasing layers of chondrocytes. That finding was in accordance with previous *in vitro* studies showing that the hormone, in a dose-dependent manner, had a stimulatory effect on the cell production of proteoglycans and type II collagen[33,34,38]. Further, calcitonin significantly reduced the collagenase activity in OA articular cartilage explants[27,34]. Regarding the subchondral bone morphology, smaller osteophytes, with a tendency of reossification, and cystic formations were observed in both *calcitonin groups* than in the placebo groups. Those findings supported previous knowledge that salmon calcitonin improved bone turnover, the structure of trabeculae and the quality of bone matrix deposition[28–30]. The number of chondrocytes in areas with mild OA lesions was normal in both *calcitonin groups* or a hypercellularity was noticed indicating regenerative activity. Additionally, nuclei and matrix staining in both *calcitonin groups* revealed significantly higher metabolic activity when compared with the placebo group. That finding supports the above-mentioned role of calcitonin that stimulates the production of proteoglycans and type II collagen[33,34,38].

The above reported *in vitro* and *in vivo* observations and many recent pre-clinical studies conducted mostly in animal OA models offer the strongest arguments for calcitonin as a potential agent for the treatment of human osteoarthritis. The macroscopic and microscopic findings of this study support the biochemical results of previous studies and indicate that the administration of salmon calcitonin in rabbits inhibits the progress of experimentally induced osteoarthritis (chondro-protective role of calcitonin). Furthermore, thickening of hyaline cartilage layers, healing of ulcerations, smaller size of osteophytes than in placebo group, and restoration of chondrocytes' metabolic activity demonstrate the regenerative effect of calcitonin on the cartilage and subchondral bone (chondro-therapeutic role). Conclusively, calcitonin treatment contributed to the observed reduction in the score of the OA microscopic lesions indicating a clear chondro-protective and chondro-regenerative roles against post-traumatic arthritis.

There are some limitations to this study. Firstly, our experimental model produced chondral changes induced after an acute traumatic event such as CCL transection. Therefore, we should not compare these changes with the slowly progressive ones in degenerative arthritis. CCL transection results in a true instability-induced OA lesion that mimics osteoarthritis occurring naturally in humans following traumatic injury[39–41]. Secondly, given the

Table VI
Comparison of the distribution of chondrocytes between groups. Median, minimum and maximum values are according to modified Mankin grading system.[18]

	Median value [minimum and maximum values]	
	Prophylactic-stage calcitonin vs placebo	Therapeutic-stage calcitonin vs placebo
Sites with mild OA changes	0/2 [0–3/0–3], $P = 0.000$	2/2 [0–3/2–3], $P =$ NS

OA: osteoarthritic.

Table VII
Comparison of the metabolic activity of the chondrocytes between groups. Increased metabolic activity was noticed in the calcitonin groups. Percentages express the number of specimens showed positive staining in each group

	Prophylactic-stage group			Therapeutic-stage group		
	Calcitonin (%)	Placebo (%)	*P* value	Calcitonin (%)	Placebo (%)	*P* value
PAS (positive)	56.4	26.7	0.008	73.3	43.3	0.03
Alcian blue (positive)	61.5	28.9	0.004	76.7	50	0.06

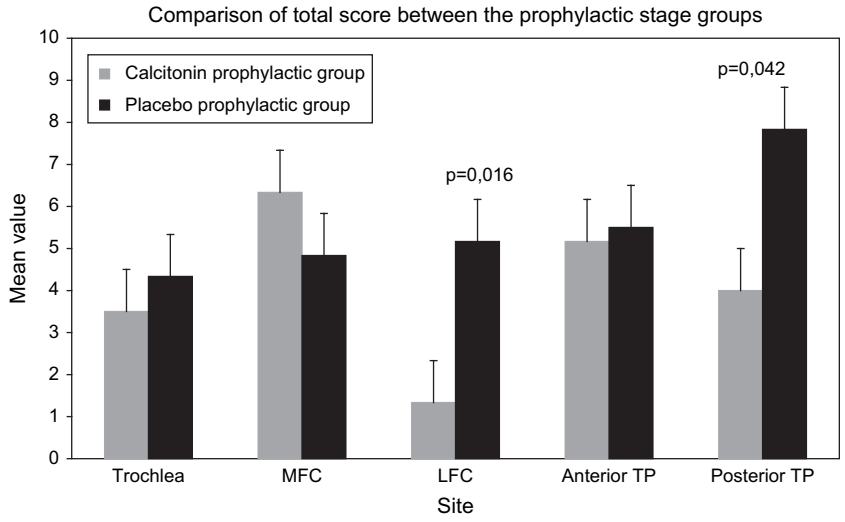

Fig. 5. In the *prophylactic-stage groups*, the calcitonin effect was significantly greater on the lateral femoral condyles (site III) and the posterior half of tibial plateau (site V).

marked differences in anatomy, biomechanics and kinetics of the rabbit and human knee[37,42,43], we should comment on the applicability of this model, in which the most striking macroscopic changes occurred on the trochlea, rather than in the tibiofemoral compartment, as is usual in human osteoarthritis. Another consideration is that it might be improper to draw conclusions on the metabolic activity of the chondrocytes based only on histochemical data. Isotopic studies or studies of synthesis or degradation of cartilage matrix macromolecules would also be appropriate for such interpretations. The last would clarify if the observed cartilage thickening is a part of a regenerative process or the first phase of a progressive arthritis. However, we correlated our macroscopic and microscopic results with the biochemical findings of other experimental studies. Finally, a reader of this study could say that we lump together changes of degeneration and regeneration by providing a total score as an index of histological severity of osteoarthritis and that it would be better to examine these microscopic parameters separately. Since the natural history of induced osteoarthritis includes both degenerative and regenerative processes[36], we thought that the total score of all the above-mentioned microscopic parameters would be more representative of the final effect of salmon calcitonin on the OA cartilage and subchondral bone.

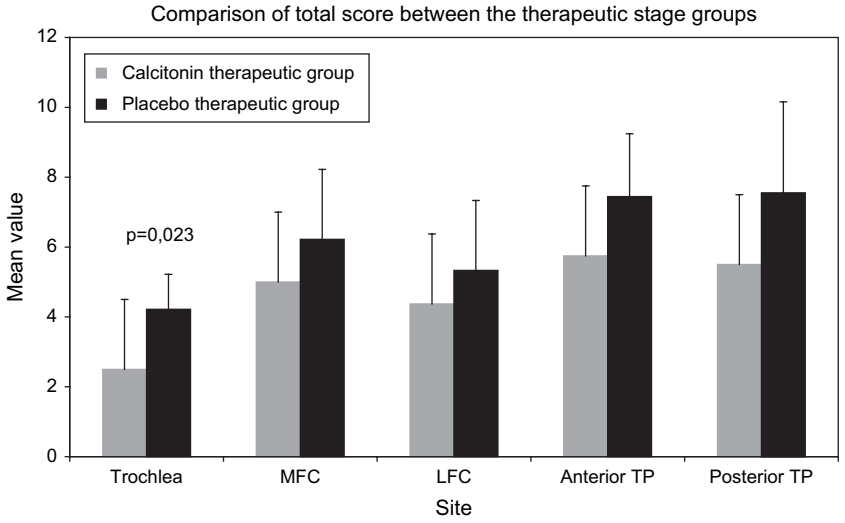

Fig. 6. In the *therapeutic-stage groups*, the calcitonin effect was significantly greater on the femoral trochlea (site I).

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